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Undernutrition—Looking Within for Answers

David A. Relman

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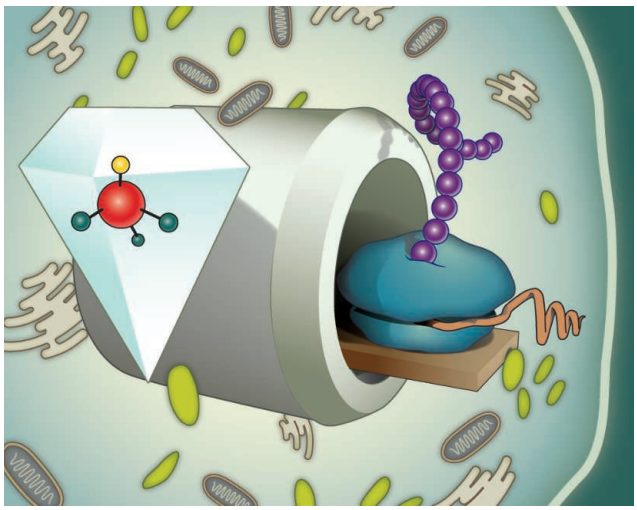

The future of live-cell imaging. In this cartoon of a possible future application of a diamond-based nanoscale MRI-machine, a single NV inside a diamond nanocrystal is used to image a ribosome in the act of translation inside a live cell. Mamin *et al*. and Staudacher *et al*. report a first step toward such a machine by demonstrating detection of protein-sized volumes of nuclear spins under ambient conditions.

nuclear spins. The magnetometer consists of a single nitrogen-vacancy (NV) color center in diamond; it can operate at room temperature in almost any environment (*6*, *7*). Previously, NVs implanted just below the surface of a bulk diamond have been used to demonstrate near-single-electron spin detection and crude one-dimensional imaging for electron spins outside of the diamond crystal (*8*). However, imaging of nuclei will be required to observe the structure and conformations of individual molecules under ambient conditions.

By implementing different advanced noise suppression techniques, Mamin *et al*. and Staudacher *et al*. have succeeded in using near-surface NVs to detect small volumes of proton spins outside of the diamond crystal. Both authors conclude that their observed signals are consistent with a detection volume on the order of $(5\ \text{nm})^3$ or less. This sensitivity is comparable to that of the cryogenic MRFM technique and should be adequate for detecting large individual protein molecules. Both groups also project much smaller detection volumes in the future by using NVs closer to the diamond surface. Staudacher *et al*. expect to improve sensitivity by using the NV to spin-polarize the nuclei. Mamin *et al*. project that sensitivity may eventually approach the level of single protons, provided that the NV coherence time can be kept long enough.

To confirm that the observed signals indeed arose from spins outside of the diamond, both groups cleaned the surface to verify that the magnetic proton signals disappeared. This was necessary as single nuclear spins were already detected up to a few nanometers distance inside the diamond lattice (*9*), but most of the envisioned applications require probing spins in the external environment.

Mamin *et al*. also observed the temporal evolution of the probed nuclear spins. This is only one small step from a fully functional nano-MRI, because in conventional MRI, images are acquired by recording the time evolution in the presence of applied magnetic field gradients.

The two approaches have different strengths. Mamin *et al*. manipulate the sample spins with an additional radiofrequency field; this approach allows complex protocols of classic NMR to be implemented easily. In contrast, Staudacher *et al*. use passive spectroscopy, which does not require an additional radiofrequency field.

If magnetic impurities on the diamond surface can be sufficiently controlled, it should be possible to image subnanometer volumes of nuclear spins. In this case, single-molecule dynamics like protein folding or the action of catalysts in chemical reactions might be observable in real time. Furthermore, if these techniques can be extended to NVs in nanodiamonds, such processes might be observable in more interesting ambient environments like living cells (see the figure). In contrast to competing techniques like fluorescence resonance energy transfer, the NV could accomplish this task with a larger dynamic range and without the need for separate labels at each site to be monitored. Such capability would substantially enhance our understanding of structural biology and biomolecular processes, as well as numerous additional applications.

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MICROBIOLOGY

Undernutrition—Looking Within for Answers

David A. Relman[1,2]

A link between the gut microbiota and conditions of undernutrition point to possible therapeutic interventions.

Undernutrition—a condition resulting from inadequate intake or assimilation of nutrients—underlies more than one-third of all deaths worldwide in children younger than 5 years of age (*1*). Enteric infectious disease and undernutrition exacerbate and perpetuate each other by means of impaired innate and adaptive immune responses. Together they produce an insidious condition called environmental enteropathy in which damaged gut mucosal architecture and function are associated with malabsorption, dysregulation of mucosal permeability, and inflammation. This vicious cycle leaves approximately one-fifth of children in the world stunted, and leads to a wide range of continuing health and developmental problems, possibly including cognitive impairment and metabolic syndrome in adulthood (*2*). Maternal malnutrition brings about malnutrition in their children [shockingly, nearly one-third of women in Bangladesh (*3*), as well as in other countries with similar levels of poverty, suffer from this condition]. These multiple, interwoven, and mutually reinforcing factors pose major challenges for efforts to address this situation. On page 548 of this issue, Smith *et al*. (*4*) show, with the use of an animal model, that the gut microbiota is involved in propagating a form of severe

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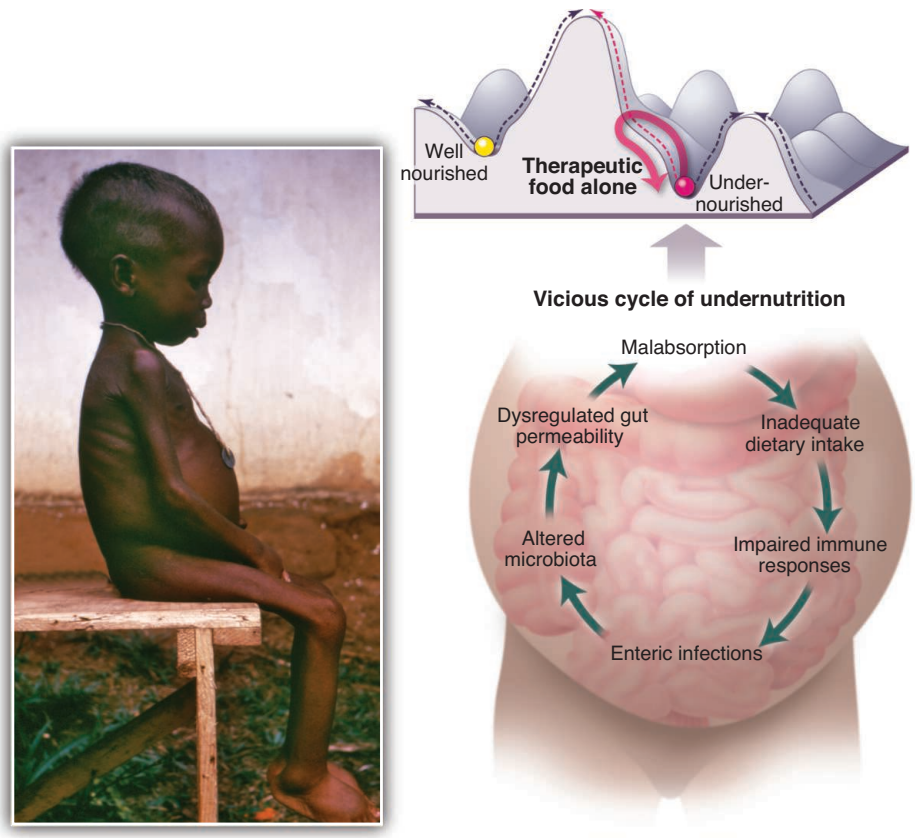
acute undernutrition called kwashiorkor (see the figure). Like previous data linking the microbiota with obesity (*5*), these findings are remarkable. They also provide hope, in that by understanding the role of the gut microbiota in undernutrition, we can devise new ecologically inspired strategies for correcting this problem.

Smith *et al*. recruited 317 twin pairs in rural southern Malawi and followed them with periodic fecal sampling until they were 3 years of age. They selected 13 same-gender twin pairs that were discordant for kwashiorkor and 9 twin pairs that were well-nourished, and then characterized their fecal microbiomes. Twin pairs with kwashiorkor were treated with ready-to-use "therapeutic food" for a mean duration of 9 weeks. By relying on the same-gender mono- and dizygotic twin pairs (genetically identical twins and fraternal twins, respectively), they could isolate the impact of genetics and household environment.

A key finding of Smith *et al*. is that the overall gene content (which generally predicts functions) of the fecal microbiota in the kwashiorkor-afflicted children, in contrast to that of the well-nourished children, fails to develop with increasing age, even with the use of therapeutic food. Although there was an initial change in the gene content of the microbiota with initiation of therapeutic food, it was not sustained. The latter finding is disappointing but not surprising, given the many other determinants (besides diet) of the "fitness landscape" of the gut (that is, the likelihood of community stability under a variety of ecosystem conditions). To clarify the role of the gut microbiota in determining host nutritional state, as well as to tease apart the contributions of diet and microbiota, Smith *et al*. collected fecal microbial communities from three discordant twin pairs at the time of kwashiorkor diagnosis and transferred them into germ-free mice. The mice were fed a diet based on the typical foods of these human donors in Malawi. Two of the three groups of mice that received microbiota from a kwashiorkor-affected twin lost weight, but only if they were fed a diet based on the typical foods of these Malawian donors. Mice that received microbiota from a healthy sibling, or fed standard mouse chow, did not lose weight. A subsequent 2 weeks of treatment with therapeutic food produced weight gain in all groups of mice, and a return to the Malawian diet again led to weight loss in the recipients of the kwashiorkor microbiota. Meaningful changes in fecal taxonomic, gene, and metabolite content accompanied these transplantations and dietary shifts in the recipient mice. Abundance of bifidobacteria and lactobacilli, as well as amounts of essential and nonessential amino acids in fecal samples, increased in mice that received kwashiorkor microbiota during administration of therapeutic food, albeit temporarily. Notably, in mice harboring a kwashiorkor microbiota that were fed a Malawian diet, analysis of urinary metabolite abundance and microbiome composition revealed inhibition of the tricarboxylic acid cycle (indicative of impaired cellular metabolism and energy production of the host). In general, the data reinforce the concept of host-microbiota cometabolism; however, additional human subjects with kwashiorkor and other forms of undernutrition should be studied to confirm these findings.

The findings of Smith *et al*. are a reminder that isolated factors, such as individual microbes or even entire microbial communities, alone cannot explain complex phenotypes such as undernutrition, and that correction of these phenotypes will require a coordinated system-wide approach. Modification of the gut microbiota may improve the nutritional state of individuals with kwashiorkor, but accomplishing this will not be easy. Genetic determinants of the host that impact microbiota composition and function are just beginning to be identified (*6*, *7*). For example, murine angiotensin I converting enzyme 2 (ACE2), a key regulatory enzyme of the renin-angiotensin system (which controls blood pressure and fluid balance), was unexpectedly linked with tryptophan absorption in the small intestine and activation of the signaling protein mammalian target of rapamycin (mTOR), which in turn triggered the expression of small antimicrobial peptides in the gut (*8*). Further, tryptophan deficiency led to diminished antimicrobial peptide expression, altered microbiota taxonomic composi-



Nutritional state. A child suffering from undernutrition (the condition kwashiorkor) is shown. The diagram (lower right) shows the interplay between factors that sustain a state of undernutrition. An ecological fitness landscape of the human-microbial ecosystem (upper right) illustrates how there are deep stability "domains"(or "basins of attraction") associated with undernutrition and overnutrition; other stability domains are associated with more healthy nutritional states. Therapeutic food alone is insufficient to induce a sustained shift in the gut ecosystem state (solid red arrow) that would cause an undernourished individual to achieve a more healthy nutritional state (dashed red arrow). Correction of this self-propagating condition will require multiple, concurrent measures to remold the environmental landscape of the host gut, thereby inducing a shift toward a gut microbiome with more efficient nutrient utilization (energy harvest) and fewer intrinsic proinflammatory properties.

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tion, and an enhanced propensity for inflammation in the colon. It could be that human genetic variation in ACE2 function or expression is associated with distinct physiological states of the gut epithelium as well as with distinct microbiota compositions in the gut; these variable factors may affect the absorptive function of the epithelium, with implications for the host nutritional state.

The history of environmental exposures and health status of a woman before and during pregnancy affect the intrauterine development of her child, as well as her own microbiota—which in turn is transmitted to her child at birth. In the setting of this inherited history, early postnatal exposures to enteric pathogens with subsequent inflammatory responses in the gut, altered immune system development, and damaged intestinal barrier function, as well as antibiotic use, insufficient food intake, and macro- and micronutrient deficiencies, all conspire to produce an ecological fitness landscape in the host with strong undernutrition-associated stability domains, and high barriers preventing shifts to health-associated states. Signaling within the microbiota, and between it and the host, can perpetuate this pathologic state.

Thus, the challenge in ecological terms is to devise a strategy for altering the fitness landscape in such a manner as to favor transitions to health-associated stable states (*9*). Indeed, there are multiple possible points of attack. Possible therapeutic approaches include delivery of defined microbial strains—either alone or as collectives—key nutrients for host and microbiota, molecules that exploit microbiota-host signaling or trigger self-regenerative responses in the gut mucosa and epithelia, and modulators of mucosal immune function. The degree to which these approaches should be individualized for different subjects and clinical settings is not yet clear, nor are the means by which the resulting beneficial effects can be made to persist. But we are well on our way toward the kind of detailed understanding of the human microbial ecosystem and its molecular and physiological features that will be necessary to enable the design and testing of these approaches.

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PHYSICS

Measuring Mass in Seconds

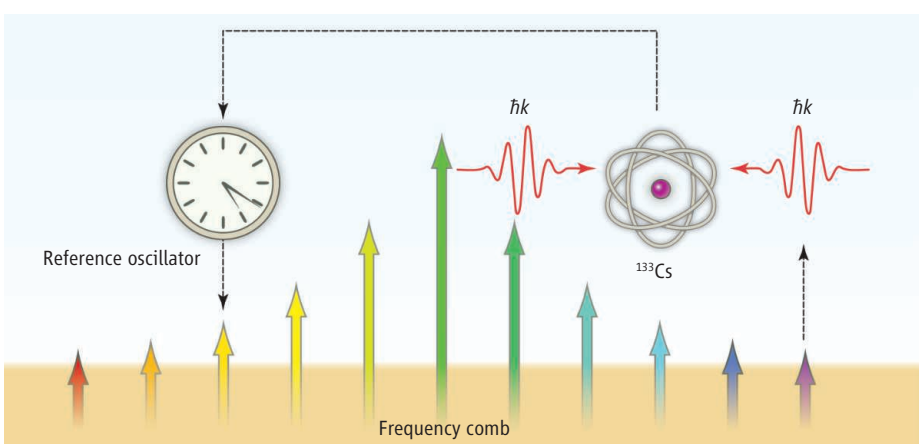
An atom interferometer and an optical frequency comb measure the Compton frequency of a cesium atom, creating a "clock" that weighs atoms.

John E. Debs, Nicholas P. Robins, John D. Close

For centuries, humans have measured time by counting oscillations of highly regular periodic motion—the Sun, a pendulum, or a quartz crystal, for example. During the past 50 years, we have chosen to use the electromagnetic oscillations, which drive absorption in an atom—a highly stable and universal frequency reference. Such atomic clocks define the SI second via an atomic resonance in cesium (*1*). The second is the most precisely defined physical unit. Although it may seem obvious now, making the leap from performing precise spectroscopy on the atomic structure of cesium to using its atomic structure as a precise reference to stabilize other oscillators was profound. On page 554 of this issue, Lan *et al*. (*2*) make an analogous distinction between performing momentum-spectroscopy on a recoiling atom, and using that spectroscopy to stabilize an oscillator, effectively locking a clock to the mass of a particle. This result has important implications for fundamental physics and precision measurement, and could play a role in a new definition of the kilogram.

Einstein's theory of relativity gives us the equivalence of energy and mass, while quantum mechanics links energy and frequency. Thus, there is, at least theoretically, a direct link between a particle's rest mass and time, expressed by the Compton frequency $\omega_c = mc^2/\hbar$. Here, m is the rest mass of the particle, c is the speed of light, and $\hbar$ is Planck's constant h divided by 2π. For an atom such as cesium, ω_c is on the order of 10^{25} Hz—an extremely high frequency to attempt to access experimentally. Strictly speaking, because a quantum state evolves at a rate directly proportional to its energy, ω_c will contribute to its evolution.

A frequently appearing quantity in quan-



Locking a "Compton clock." A schematic representation of the experiment of Lan *et al*., in which an atom interferometer measures the recoil frequency of a cesium-133 atom with lasers locked to a known frequency-comb line. This method ensures that the recoil frequency is a defined fraction of the Compton frequency of the atom. This signal stabilizes a reference oscillator, which is the reference for all frequencies in the system, including the frequency comb itself. In this way, a clock is locked to the mass of an atom.

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